REMARKS

Claims 1, 4-7 and 14 are pending. The amendment to claim 1 is supported by canceled claim 13. No new matter has been added.

Claim Objections

Claim 1 is objected to for informalities.

Claim 1 has been amended as suggested by the Examiner.

Claim Rejections

Claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite. (Office Action p.3)

Claim 1 has been amended as suggested by the Examiner for clarity.

Claims 1, 4 – 6 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mejia et al. (Genomics 70(2): 165 – 170, 2000) in view of Waye et al. (Mol and Cell. Biol. 6(9):3156-3165, 1986), Ikeno et al. (Human Mol. Gen 3(8):1245 – 1257, 1994) and Perkins et al. (US 2003/0119104A1)). (Office Action, p. 15).

Claim 1 has been amended with the subject mater of claim 13, which distinguishes the claimed invention over the combination of the prior art, as will be shown herein. The claimed invention is based on a novel production method of a mammalian artificial chromosome (MAC) that includes a first, second and third vector. The second vector comprises an insertion sequence, wherein the insertion sequence is a loxP site, a FRT site, or a sequence obtained by partial modification of a loxP site or a FRT site and has a function for inserting the sequence of interest, and an insulator sequence. By using such a second vector, it is possible to construct a general purpose MAC, to which a predetermined sequence can be inserted later.

None of the references, when combined, teach the method comprising the steps and elements as claimed. In particular, the Mejia reference does not teach a second vector that comprises an insertion sequence, as claimed, and an insulator sequence. The Mejia reference

simply teaches *de novo* generation of a HAC construct with a HPRT genomic insert, as shown in Figure 1 (map of the HAC construct). The rejection, on p.7, confirms that Mijia does not teach the method being performed in mammalian cells, nor that the vector comprises an insulator sequence. Furthermore Perkins does not teach insertion sequences which is a loxP site, a FRT site, as claimed. Wayne was cited for the sequence of the human chromosome 17 centromere and Ikeno was cited for teaching a consensus nucleotide sequence from human chromosome 21 alphoid which repeats. Neither Mejia nor any of the references teaches or suggests a general purpose MAC. Furthermore the combination of references fail to teach the claimed insertion sequence or insulation sequence.

In short, none of the secondary references cure the defects of the Mejia reference. Combined, the Waye, Ikeno and Perkins references do not cure the flaws of the Mejia reference, nor do they teach or suggest a production method of a mammalian artificial chromosome as instantly claimed.

Moreover, the MAC of the instant invention has an insulator sequence for the purpose of **promoting the expression of a gene to be introduced later**, and it was found by the inventors that, surprisingly, **both of** the efficiency of gene transfer into the mammalian artificial chromosome **and the efficiency of the expression of gene were** enhanced. This point is disclosed in the specification in paragraphs [0009] and [0160] of the published application:

> [0009] Furthermore, in the production of a mammalian artificial chromosome having a gene insertion site, when a mammalian artificial chromosome was constructed by inserting an insulator sequence for the purpose of promoting the expression of gene to be introduced later, surprisingly, the efficiency of gene transfer into the mammalian artificial chromosome was enhanced. In other words, it was found that the use of the insulator sequence makes it possible to produce efficiently mammalian artificial chromosome having a target gene.
>
> [0160] It is preferable that the second vector to be used in the present invention has an insulator sequence. Herein, the insulator sequence is a base sequence characterized by exhibiting an enhancer blocking effect (expressions of neighboring genes are not affected by each other) or a chromosome boundary

effect (a region assuring the gene expression and a region suppressing the gene expression are separated with each other). It is expected that the use of the insulator sequence promotes the expression of a target gene contained by a mammalian artificial chromosome. On the other hand, as shown in Examples mentioned below, when the above-mentioned inserting sequence such as loxP, etc. is used, if the insulator sequence is used together, it was found that the introduction rate of the target gene into the mammalian artificial chromosome was increased. Thus, when the insulator sequence is used, the effect of increasing the rate of introducing genes into the mammalian artificial chromosome can be exhibited. Therefore, it is possible to construct effectively and more certainly the mammalian artificial chromosome that holds the target gene. Usable insulator sequences are not particularly limited. It is possible to use not only an insulator, which has been identified as an insulator, but also a sequence obtained by providing modification for the sequence as long as the expected effect (the increase in promoting the expression of target gene or the increase in the gene introduction efficiency) is not reduced. A plurality of insulator sequences may be used together. When a plurality of insulator sequences are used one kind of insulator sequence may be used or plural kinds of insulator sequences in combination may be used. Note here that human B globin HS1 to 5, chicken β-globin HS4, Drosophila gypsy retrotransposon, sea urchin 5' flanking region of arylsulfatase, blocking element α/d of human T-cell receptor α/d, repeat organizer of Xenopus 40S ribosomal RNA gene, and the like, have been known as insulator sequence.

In contrast, human artificial chromosomes (HACs) that are heretofore known in the art have the capacity to accommodate a large transgene, but are generated ***de novo*** from a precursor construct with both the transgene and an alphoid array. Thus, the art contemplates neither a general purpose MAC nor the use of an insulator sequence, both requirements of the invention now claimed.

It is respectfully requested that the rejection be withdrawn.

Claims 1 and 7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mejia et al. (Genomics 70(2): 165 – 170, 2000) in view of Waye, Ikeno, and Perkins, and further in view of Bokkelen (US 5,695,967). (Office Action, p. 17).

Claim 1 has been amended with the subject matter of claim 13, and as previously explained is not obvious in light of Mejia in view of Waye, Ikeno and Perkins. Bokkelen does not cure the deficiencies of the combination of the other art because it no where discloses or even suggests an insertion sequence being a loxP site, a FRT site, etc. and a insulation sequence. Therefore claims 1 and 7 are not rendered obvious by the combination of disclosures.

It is respectfully requested that the rejection be reconsidered and withdrawn.

Claims 1 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mejia et al. (Genomics 70(2): 165 – 170, 2000) in view of Waye, Ikeno, Perkins, and Bokkelen, and further in view of Cooke (WO 00/18941). (Office Action, p. 19).

Cooke was cited for disclosing a method of making mammalian artificial chromosomes. However the references cannot compensate for the deficiencies of the other references because it does not disclose an insertion sequence being a loxP site, a FRT site, etc. and a insulation sequence. Therefore the combination of references fails to make a *prima facie* rejection of obviousness.

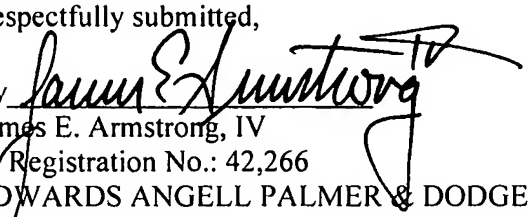
It is respectfully requested that the rejection be reconsidered and withdrawn.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 04-1105.

Dated: January 13, 2009

Respectfully submitted,

By 

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